

Application Serial No. 10/630,968
Amendment dated 28 January 2011
Reply to Office Action dated 16 December 2010

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

Claims 1-2 (canceled).

Claim 3 (currently amended): The method of claim [[1]] 33, wherein the mammalian promoter is a Pol III promoter.

Claim 4 (original): The method of claim 3, wherein the Pol III promoter is a mammalian U6 promoter.

Claim 5 (original): The method of claim 4, wherein the U6 promoter is a human U6 promoter.

Claim 6 (currently amended): The method of claim [[1]] 33, wherein the sequence encoding the terminator sequence comprises a sequence of about 4-6 deoxyadenosines.

Claim 7 (original): The method of claim 6, wherein the sequence encoding the terminator sequence comprises a sequence of 6 deoxyadenosines.

Claim 8 (currently amended): The method of claim [[1]] 33, wherein the second oligonucleotide primer further comprises a tag sequence to identify functional siRNA encoding sequences.

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Claim 9 (original): The method of claim 8, wherein the tag sequence further comprises a restriction site useful for cloning.

Claims 10-16 (canceled).

Claim 17 (currently amended): The method of claim [[1]] 36, further comprising the step of transfecting a mammalian cell *in vitro* with the first amplified mammalian promoter-containing siRNA expression cassette and with the second amplified mammalian promoter-containing siRNA expression cassette, wherein an siRNA molecule is the sense strand of the double stranded siRNA molecule and the antisense strand of the double stranded siRNA molecule are expressed in the transfected cell, whereby a double stranded siRNA molecule is produced in the transfected cell.

Claim 18 (canceled).

Claim 19 (original): The method of claim 17, wherein one or more of the oligonucleotide primers are modified.

Claim 20 (original): The method of claim 19, wherein one or more of the oligonucleotide primers are modified by phosphorylation.

Claim 21 (currently amended): The method of claim 17, further comprising the step of screening for a target site on mRNA sensitive to the expressed double stranded siRNA molecule produced.

Claims 22-32 (canceled).

Claim 33 (new): An amplification-based method for producing a mammalian promoter-containing siRNA expression cassette, comprising:

(i) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells;

(ii) adding a first oligonucleotide primer to the reaction mixture, wherein the first oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid;

(iii) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes (1) either a sense sequence of a double stranded siRNA molecule or an antisense sequence of the double stranded siRNA molecule and (2) a terminator sequence; and

(iv) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (a) annealing the primers to the complementary strands of the double stranded nucleic acid, (b) extending the annealed primers to produce extension products, (c) denaturing the extension products and (d) repeating the polymerase chain reaction amplification steps a sufficient number of times to produce an amplified product comprising the mammalian promoter-containing siRNA expression cassette,

wherein the mammalian promoter-containing siRNA expression cassette comprises (1) the mammalian promoter, (2) either the sense strand or the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence.

Claim 34 (new): The method of claim 33, wherein the amplification product is purified.

Claim 35 (new): The method of claim 33, further comprising cloning the amplified product comprising the mammalian promoter-containing siRNA expression cassette into a cloning vector.

Claim 36 (new): The method of claim 33, wherein two polymerase chain reaction amplifications are performed, wherein a first polymerase chain reaction amplification is performed to produce a first amplified product comprising a first mammalian promoter-containing siRNA expression cassette comprising (1) the mammalian promoter, (2) the sense strand of the double stranded siRNA molecule and (3) the terminator sequence, and wherein a second polymerase chain reaction amplification is performed to produce a second amplified product comprising a second mammalian promoter-containing siRNA expression cassette comprising (1) the mammalian promoter, (2) the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence.

Claim 37 (new): The method of claim 36, wherein the first and second amplification products are purified.

Claim 38 (new): The method of claim 36, further comprising cloning the first amplified product comprising the first mammalian promoter-containing siRNA expression cassette into a cloning vector and cloning the second amplified product comprising the second mammalian promoter-containing siRNA expression cassette into a cloning vector.